

## Product Information

**PKC $\iota$ , active, GST-tagged, human  
PRECISIO® Kinase  
recombinant, expressed in Sf9 cells**

Catalog Number **K4393**  
Lot Number 041M0990  
Storage Temperature  $-70\text{ }^{\circ}\text{C}$

Synonyms: PRKCI, DXS1179E

### Product Description

Protein kinase C iota (PKC $\iota$ ) is a member of the protein kinase C family of serine-threonine kinases. The amino acid sequence of PKC $\iota$  showed greatest homology to PKC $\zeta$ , with 72% identity overall rising to 84% in the catalytic domain. PKC $\iota$  has been implicated in Ras signaling and is a critical downstream effector of oncogenic Ras in the colonic epithelium. Transgenic mice expressing constitutively active PKC $\iota$  in the colon are highly susceptible to carcinogen-induced colon carcinogenesis.<sup>1</sup>

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 002740. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~98 kDa

Purity:  $\geq 70\%$  (SDS-PAGE, see Figure 1)

Specific Activity: 564–764 nmole/min/mg (see Figure 2)

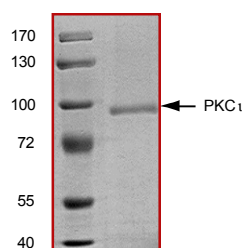
### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

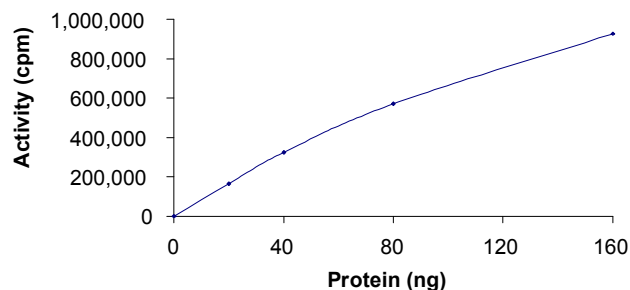
### Storage/Stability

The product ships on dry ice and storage at  $-70\text{ }^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70\text{ }^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Lot Number 041M0990:  
>90% (densitometry)



**Figure 2.**  
Specific Activity of Lot Number 041M0990:  
664 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl<sub>2</sub>, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with water.

Kinase Solution – Dilute the active PKC $\iota$  (0.1  $\mu\text{g}/\mu\text{l}$ ) with Kinase Dilution Buffer to the desired concentration.

**Note:** The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PKC $\iota$  kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200  $\mu\text{l}$  aliquots at  $-20\text{ }^{\circ}\text{C}$ .

$\gamma$ - $^{32}\text{P}$ -ATP Assay Cocktail (250  $\mu\text{M}$ ) – Combine 5.75 ml of Kinase Assay Buffer, 150  $\mu\text{l}$  of 10 mM ATP Stock Solution, 100  $\mu\text{l}$  of  $\gamma$ - $^{32}\text{P}$ -ATP (1 mCi/100  $\mu\text{l}$ ). Store in 1 ml aliquots at  $-20\text{ }^{\circ}\text{C}$ .

Substrate Solution – Dissolve the synthetic peptide substrate (KRREILSRPSYR) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

#### Kinase Assay

This assay involves the use of the  $^{32}\text{P}$  radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active PKC $\iota$ , Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The  $\gamma$ - $^{32}\text{P}$ -ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20  $\mu\text{l}$ :
  - 10  $\mu\text{l}$  of Kinase Solution
  - 7.5  $\mu\text{l}$  of Substrate Solution
  - 2.5  $\mu\text{l}$  of PKC lipid activator (0.5 mg/ml phosphatidylserine and 0.05 mg/ml diacylglycerol in 20 mM MOPS, pH 7.2, containing 1 mM  $\text{CaCl}_2$ ). Sonicate lipid for 1 minute prior to use.
3. Set up a blank control as outlined in step 2, substituting 7.5  $\mu\text{l}$  of cold water ( $4\text{ }^{\circ}\text{C}$ ) for the Substrate Solution.
4. Initiate each reaction with the addition of 5  $\mu\text{l}$  of the  $\gamma$ - $^{32}\text{P}$ -ATP Assay Cocktail, bringing the final reaction volume to 25  $\mu\text{l}$ . Incubate the mixture in a water bath at  $30\text{ }^{\circ}\text{C}$  for 15 minutes.

5. After the 15 minute incubation, stop the reaction by spotting 20  $\mu\text{l}$  of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.
6. Air dry the pre-cut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of  $\sim 10$  minutes each.
7. Set up a radioactive control to measure the total  $\gamma$ - $^{32}\text{P}$ -ATP counts introduced into the reaction. Spot 5  $\mu\text{l}$  of the  $\gamma$ - $^{32}\text{P}$ -ATP Assay Cocktail on a pre-cut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

#### Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$\text{SR} = \frac{\text{cpm of } 5\ \mu\text{l of } \gamma\text{-}^{32}\text{P}\text{-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

$$\begin{aligned} &\text{cpm} - \text{value from control (step 7)} \\ &\text{nmole} - 1.25\ \text{nmole (} 5\ \mu\text{l of } 250\ \mu\text{M ATP} \\ &\text{Assay Cocktail)} \end{aligned}$$

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{\text{SR} \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)

$\Delta\text{cpm}$  = cpm of the sample – cpm of the blank (step 3)

25 = total reaction volume

20 = spot volume

T = reaction time (minutes)

E = amount of enzyme (mg)

#### **References**

1. Murray, N.R. et al., Protein kinase C iota is required for Ras transformation and colon carcinogenesis *in vivo*. *J. Cell Biol.*, **164**, 797-802 (2004).

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